ENGINEERING BONY HYBRID ORGANS IN VITRO

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ABSTRACT

Hybrid bony organs containing mineralized tissue, marrow and microcirculatory compartments could provide extremely novel and life-saving biosensors and tissue replacements. Previous progress in engineering distinct elements of bone suggests this more complex goal is feasible, but the challenges of integrating these elements into a single organ remain to be addressed. We are exploiting recent advances in stem cell biology, materials sciences, and microfabrication to create complex, multi-component bony organs. Materials presenting nanoscale-organized cell adhesion ligands and localized availability of growth factors have been developed, based on a combination of theoretical and experimental studies, to control the proliferation and differentiation of multipotent stem cells. This control is being exploited to create tissues *in vitro* within microfluidic systems that compartmentalize the cells and regulate their access to nutrients and waste exchange. Hematopoietic stem cells (HSCs) are being subsequently introduced into the engineered bony organs in an effort to establish functional blood cell forming organs. These engineered bone marrow organs could provide an unusually sensitive physiological biosensor for the presence of various toxic agents. Moreover, these organs could be customized for transplantation into individual soldiers requiring reconstitution of their bone marrow. The technologies developed to create these organs may also provide a template for the engineering of other complex, hybrid organs comprised of multiple cell types (e.g., liver, neural tissues) that would be useful for directly addressing trauma or promoting regeneration of damaged tissues and organs.

1. INTRODUCTION

Bone is a complex, multifunctional system comprised of multiple tissue types, and the loss or failure of specific components can lead to a variety of serious or fatal consequences. Mineralized bony tissue has outstanding mechanical strength, and maintains and protects the soft tissues of the body. Trauma often results in the requirement for mineralized tissue replacement. Contemporary approaches to replace the mechanical functions of bone, including autologous or allogeneic bone transplantation and implantation of metallic or reinforced polymeric hardware, have significant limitations. The marrow component of bone is also itself a complex tissue, consisting of the hematopoietic system and the bone marrow stroma, and is also a target of noxious agents potentially experienced by soldiers in the field. Because large numbers of blood cells must be produced every day by highly proliferative hematopoietic progenitors, the bone marrow is more sensitive than any other tissue to cytotoxic environmental agents. The limitations of current therapies to treat bone damage have led to significant efforts to engineer or regenerate bony tissue using delivery of inductive proteins or transplantation of selective cell population, and recent advances in stem cell biology suggest it may be possible to recreate the various components of bone from multipotent cells resident in bone marrow (Alsberg et al., 2001).

To promote bone regeneration and long-term hematopoietic stem cell self-renewal, it will likely be necessary to provide a complex combination of signals in an appropriate 3-dimensional environment. Although it is well accepted that 3-D tissue constructs have characteristics that differ greatly from typical 2-D cell culture (Silva and Mooney, 2004), the mechanism of the difference is not well understood or controlled. Possible explanations for different cell behaviors include differences in: (i) 3-D arrangement of adhesive sites and in contact between neighboring cells, or (ii) the local chemical concentrations resulting from mass transport effects –that is, lower oxygen/nutrient concentrations and/or higher autocrine/growth factor concentration in the vicinity of cells resulting from the small volume and slow exchange of media around cells in 3-D matrices. Most likely it is a combination of these factors that makes 3-D engineered constructs different from 2-D culture systems.

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We propose that an interdisciplinary experimental and theoretical approach combining elements of stem cell biology, biomaterials design, and microfluidics may be used to create, in vitro, the appropriate microenvironmental niches necessary for the generation of hybrid bony organs containing the critical elements of normal bone tissue. Hydrogels used for cell immobilization in 3-D can be used present cell adhesion ligands and growth factors in specific nanoscale patterns to control the differentiation of stem cells. Multilayer soft lithography may be used to construct 3-D microscaffolds that contain distinct compartments for the various tissues in the bony organs, and integrated pumping/valving/mixing system can potentially provide microfluidic circulatory support to the different compartments.

2. RESULTS

2.1 Hematopoietic stem cell (HSC) and Mesenchymal Stem Cell (MSC) isolation

Success in this project is dependent on the development of methodologies to isolate and characterize two distinct stem cell populations – hematopoietic stem cells (HSCs) that can reconstitute the blood cell forming function of bone, and mesenchymal stem cells (MSCs) that can form mineralized tissue.

We have dramatically enhanced the ability to purify mouse hematopoietic stem cells (HSCs) by discovering that cell surface receptors of the SLAM family, including CD150, CD244, and CD48, are differentially expressed among functionally distinct progenitors (Kiel et al., 2005). This allowed us to highly purify HSCs using antibodies against a simple combination of SLAM family members. One of our major goals was to purify HSCs to the point that it is possible to reliably reconstitute the hematopoietic systems of irradiated mice by transplanting only a single cell. Using these new markers we have accomplished this goal, long-term multilineage reconstituting 45% of irradiated mice, after transplantation of single $CD150^{+}CD48^{-}CD41^{-}$ cells. Further, we have also recently demonstrated that these markers can successfully purify HSCs from old, reconstituted, or cytokine mobilized mice, and HSCs from these sources engraft relatively efficiently after transplantation (Kim et al., 2005; Yilmaz et al., 2006).

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The identification of a true MSC lags behind characterization of HSCs, but we have found that we can greatly enrich for MSC-like activity from mesenchymal cells of the bone marrow. To enrich the population of mesenchymal stem cell-like cells, rats or mice were administered 5-flurouracil (5-FU) *in vivo*. Limiting dilution analysis demonstrated that 5-FU treated bone marrow had the potential to form colony forming units-fibroblastic (CFU-F) at a 10-fold or 6-fold enrichment compared to normal bone marrow in rats, or mice, respectively. *In vivo* and *in vitro* differentiation assays supported the enrichment and purification effects. Further, we have addressed the issue that, in contrast to embryonic stem cells, MSCs have a more limited lifespan and progressively lose their "stemness" during *ex vivo* expansion, possibly due to the lack of telomerase activity. Forced expression of human telomerase reverse transcriptase (hTERT) in MSC extended their lifespan and maintained their osteogenic potential (Shih et al., 2002).

2.2 Controlling cell fate with microenvironment

A variety of environmental cues can potentially be exploited to direct cell fate, and we have made significant progress in delineating the role of nanoscale adhesion ligand organization, growth factor presentation, and cell-cell interactions in control of MSC and HSC fate.

Control Over MSCs

Cellular adhesion ligands regulate many aspects of cell phenotype, and we hypothesized the nanoscale organization of these ligands may regulate the proliferation and differentiation of bone forming cells. To investigate this possibility, alginate, a widely utilized biomaterial that can form hydrogels, was chemically modified with a peptide containing the cell adhesion sequence arginine-glycine-aspartic acid (RGD). There is a vast experimental space available to sample, though, with the variables of ligand density, nanoscale spacing, and valency. To guide the experiments, we have developed a multi-scale approach to model the patterning of adhesion ligands. The spacing of ligands within an individual polymer chain is modeled using a freely jointed chain model (Comisar et al., 2006), and the spacing of the polymer chains within the gels using a Monte Carlo packing model (Lee et al., 2004). These models have been used to predict patterns that regulate cell adhesion (Brinkerhoff et al., 2005), and the nanoscale spacing of RGD clusters in alginate gels was found to regulate the proliferation and differentiation of both preosteoblasts (Lee et al., 2004) and MSCs, and the dependency on these adhesive cues increased with the stage of cell differentiation. These results indicate that nanoscale ligand organization provides an important new variable to regulate cell proliferation and differentiation.

While adhesion ligands clearly regulate cell fate, a variety of other microenvironmental signals are also crucial. The differentiation of MSCs appears to require the signaling of specific growth factors, and this suggests a multifactorial approach will be required to design appropriate microenvironments to control stem cell fate. To address this issue, we have been developing systems to

allow for the localized transfection of cell populations in order to allow for the controlled production of growth factors by the cells. We have developed a highly efficient system, utilizing the sustained delivery of condensed plasmid DNA from polymers, for transfection that has demonstrated utility in bone formation (Huang et al., 2005a,b,c). To further understand the molecular mechanisms that control the differentiation of MSCs, we have explored whether the NF-kB and Wnt signaling pathways regulate their differentiation. We found that NF-kB negatively regulated osteoblast differentiation of mesenchymal cells induced by bone morphogenetic proteins (BMPs). Since NF-kB can be activated by multiple inducers including irradiation and toxin, our findings suggest that the inhibition of NF-kB may help to promote bone formation by these cells. In contrast to NF-kB, we have found that the Wnt signaling pathway, and Wnt-4 in particular, plays a critical role in the stimulation of MSC differentiation (Chang et al., submitted).

HSC control

To test the ability of newly formed bone ossicles to support the development of a mature hematopoietic organ from a single, purified HSC we have developed an in vivo model system of bone development. In this model, independent ossicles derived from MSCs are first developed in subcutaneous spaces in mice. Once the ossicles are fully developed, single HSCs are isolated and injected directly into the ossicles. Beginning at Week 4 post-transplant, peripheral blood of recipient mice was analyzed, and 4 of 4 recipients had high levels of donor-derived circulating hematopoietic cells in the B-cell, and myeloid lineages. These data suggest that purified HSCs can be transplanted into newly formed bony ossicles and develop into mature blood cells *in vivo*. This model will serve as an *in vivo* test of the stage of osteoblast differentiation needed to support hematopoietic cell development and serve as a guide for the engineering of hybrid bony organs *in vitro*.

Role of Vascular Cells

The vascular system clearly plays an important role in stem cell fate by its regulation of mass transport to these cells, and we have been replicating this function with our microcirculatory support system described below. However, we have also recently found a direct regulation over the fate of MSCs and possibly HSCs by vascular cells, and this signaling may also need to mimicked in our microcirculatory system. First, we have determined that endothelial cells regulate MSC differentiation through expression of BMP-2, and can enhance bone formation by these cells in vitro and in vivo (Kaigler et al., 2005). Secondly, our new ability to purify HSCs allowed us to determine that many HSCs were associated with sinusoidal endothelium in vivo (Kiel et al., 2005).

2.3 Microfluidic circulatory system

Microfluidic circulatory support of cells is a prerequisite for *in vitro* tissue engineering of bony tissues. We had developed a computerized microfluidic cell culture system using elastomeric channels and Braille displays (Gu et al., 2004; Futai et al., 2004). We have now further developed this system to enable robust incorporation of cells in 3-D environments using microchannels incorporating porous membranes and hydrogels, portable, long-term culture of stromal/support cells (Song et al., 2005; Futai et al., 2006), and experimental and theoretical characterization of microfluidic chips and cell growth (Mehta et al., 2006; Sud et al., 2006). Although stromal cells and support cells grow well in many microfluidic systems, the very small number of purified HSCs introduced in the system, along with the high sensitivity of these cells to shifts in evaporation induced osmolality shifts, carbon dioxide depletion mediated pH shifts, and to changes in oxygen tension makes it crucial to obtain a quantitative understanding of these factors to enable robust and efficient engineering of the on-chip HSC niche. More specifically, methods to reliably incorporate semi-porous polyester membranes and hydrogels for 3-D cell culture, and a method to directly interface microfluidic chips with fluorescence-activated cell sorters (FACS) to efficiently load rare sorted cells have been developed. Multiple valves in this system can be activated using one pin actuator (Gu et al., 2004; Futai et al., 2006). To guide device development, we have quantified experimentally and modeled theoretically, evaporation induced osmolality changes and transport and cell-uptake mediated changes in oxygen tension in our PDMS microfluidic devices. More specifically, we have developed a quantitative model describing the transport of soluble nutrients inside the micro-bioreactor, with the goal of using the model for the design and interpretation of data from the microbioreactor (Mehta et al., 2006). We have now shown that the model successfully describes the generation of oxygen gradients within the bioreactor and the dependency of those gradients on operating (e.g. flow rate, cell density) and design conditions (e.g. geometry), and oxygen levels in the reactor were quantified in real time using fluorescence intensity and lifetime imaging of an oxygen sensitive dye (Sud, et al., 2006). In particular, we can now design and then generate low oxygen tensions in the reactor, and this may be critical for optimal function of HSCs, which self-renew and maintain pluripotency more efficiently at lower oxygen tension (<5% *in vitro*).

CONCLUSIONS AND DISCUSSION

Significant advances have been made in a number of areas that are crucial to the ultimate goals of this project. These include dramatically advancing the field of HSC

purification, generation of immortalized human mesenchymal stem cells (MSCs), and a technique to significantly enrich for MSC activity from starting cell populations of bone marrow. The multiscale models of adhesion ligand patterning in hydrogels have been used to guide the experimental delineation of nanoscale ligand organizations that promote stem cell adhesion and expansion in vitro. Further, we have found that the differentiation of MSC populations is regulated by local growth factor signaling, and developed both models of intercellular factor signaling and materials systems to induce cells to locally express desired factors. The role of specific signal transduction pathways (NF-κB and Wnt) in MSC differentiation have been described for the first time, and are providing novel targets for control of bone formation. In studies integrating the various aspects of this project, we have found that engineered bones serve as a niche for the maintenance of HSC activity, and that vascular cells provide a direct regulation over the fate of MSCs and may be involved in HSC maintenance. Finally, we have developed a microfluidic circulatory system to enable robust, portable, long-term culture of cells. We have specifically developed hardware (e.g. circuitry for operation without laptop or power hookup), chip and software development, and medium for this reactor. A quantitative understanding of microfluidic cultures is emerging by combining experiments studying the role of perfusion variables on cell fate with mathematical models of nutrient distribution inside the devices.

On a broader scale, success in the ultimate aims of this project would have significant impact in a number of areas relevant to the Army and DoD. Because large numbers of blood cells must be produced every day by highly proliferative hematopoietic progenitors, the bone marrow is more sensitive than any other organ to cytotoxic environmental agents. This suggests that engineered bone marrow organs could provide an unusually sensitive physiological biosensor for the presence of such agents. Moreover, the engineered bone marrow organs could be customized for transplantation into individual patients requiring hematopoietic reconstitution after exposure to myeloablatic radiation or chemicals. The development of three-dimensional materials providing an appropriate combination of temporal and spatial signals to recreate all components of bony tissues, with a preprogrammed and controllable growth may also enable the replacement of large volumes of lost bone tissue using the transplantation of a small number of initial cells. This would address the single biggest challenge in tissue engineering at this time - the inability to replace large volumes of tissue. In any of these applications, a sufficient number of stem cells could potentially be obtained via a small blood or marrow sample of the individual to be treated. Further, the technologies developed to create these organs will provide a template for the engineering of other complex, hybrid organs comprised of multiple cell types (e.g., liver, neural tissues) that may be integrated into this system in the future

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REFERENCES

Alsberg E, Hill EE, Mooney DJ., 2001: Craniofacial tissue engineering. *Crit. Rev. Oral Biol. and Med.* **12**, 64-75

Brinkerhoff CJ, Linderman JJ, 2005: Integrin dimerization and ligand organization: Key components in integrin clustering for cell adhesion. *Tissue Eng*. **11**, 865-876.

Chang J, Sonoyama W, Wang Z, Jin Q, Krebsbach PH, Giannobile W, Shi S, Wang C-Y, submitted: Enhancing bone regeneration of human mesenchymal stem cells in craniofacial defects by non-canonical Wnt-4 signaling.

Comisar WA, Hsiong S, Kong HJ, Mooney DJ, Linderman JJ, 2006: Multi-scale modeling to predict ligand presentation within RGD nanopatterned hydrogels. *Biomaterials* **27**, 2322-2329.

Futai N, Gu W, Takayama S, 2004: Rapid prototyping of microstructures with bell-shaped cross-sections and its application to deformation-based microfluidic valves. *Adv. Mater*. **16**, 1320-1323.

Futai N, Gu W, Song JW, Takayama S, 2006: Handheld recirculation system and customized media for microfluidic cell culture. *Lab Chip*. **6**, 149-154.

Gu W, Zhu X, Futai N, Cho BS, Takayama S, 2004: Computerized microfluidic cell culture using elastomeric channels and braille displays. *Proc. Natl. Acad. Sci*. USA **101**, 15861-15866.

Huang Y-C. Simmons C. Kaigler D. Rice KG. Mooney DJ, 2005: Bone regeneration in a rat cranial defect with delivery of PEI-condensed plasmid DNA encoding for bone morphogenetic protein-4 (BMP-4). *Gene Therapy* **12**, 418-426.

Huang YC. Riddle K. Rice KG. Mooney DJ, 2005: Long-term in vivo gene expression via delivery of PEI-DNA condensates from porous polymer scaffolds. *Human Gene Therapy* **16**, 609-17.

Huang YC. Kaigler D. Rice KG. Krebsbach PH. Mooney DJ, 2005: Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. *Journal of Bone & Mineral Research* **20**, 848-57.

Kaigler D. Krebsbach PH. West ER. Horger K. Huang YC. Mooney DJ, 2005: Endothelial cell modulation of bone marrow stromal cell osteogenic potential. *FASEB J* **19,** 665-667.

Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ, 2005: SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121.

Kim I, He S, Yilmaz O, Kiel MJ, Morrison SJ, 2006: Enhanced purification of fetal liver hematopoietic stem cells using SLAM family receptors. *Blood* **108**, 737-744.

Lee KY. Alsberg E. Hsiong S. Comisar W. Linderman J. Ziff, R. Mooney DJ, 2004. Nanoscale adhesion ligand organization regulates osteoblast proliferation and differentiation. *Nano Letters*. **4**, 1501-1506.

Mehta K, Linderman JJ, 2006: Model-based analysis and design of microchannel reactor for tissue engineering. *Biotech. Bioeng*. **94,** 596-609.

Shi, S., Gronthos,S., Chen,S., Counter,C.M., Robey, P.G., and Wang, C-Y, 2002: Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. *Nature Biotechnology* **20**, 587-591.

Silva EA. Mooney DJ, 2004: Synthetic Extracellular Matrices for Tissue Engineering and Regeneration. *Current Topics in Developmental Biology***. 64**, 181-205.

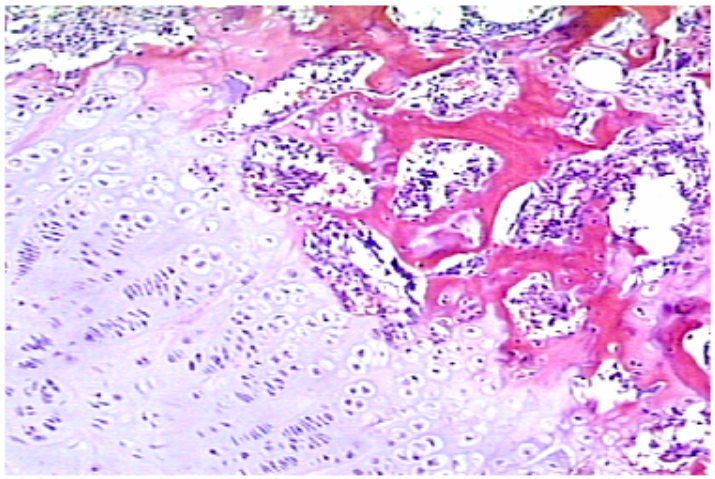
Song JW, Gu W, Futai N, Warner KA, Nor JE, Takayama S 2005: A computer-controlled circulatory support system for endothelial cell culture and shearing. *Anal. Chem*. **77**, 3993-3999.

Sud D, Mehta G, Mehta K, Linderman J, Takayama S, Mycek M, in press: Optical imaging in microfluidic bioreactors enables oxygen monitoring for continuous cell culture. *J Biomed Optics*.

Wang Z, Song J, Taichman RS, Krebsbach PH, 2006: Ablation of proliferating marrow with 5-FU allows partial purification of mesenchymal stem cells. *Stem Cells* **24**, 1573-82.

Yilmaz OH, Valdez R, Theisen B, Guo W, Ferguson D, Wu H, Morrison SJ, 2006: Pten dependence distinguishes haematopoietic stem cells from leukaemia-initating cells. *Nature* **441**, 475-482.

Engineering Hybrid Bony Organs



(Alsberg et al., *PNAS* 2002)

Engineered bony organs may provide:

- highly sensitive physiologic biosensors

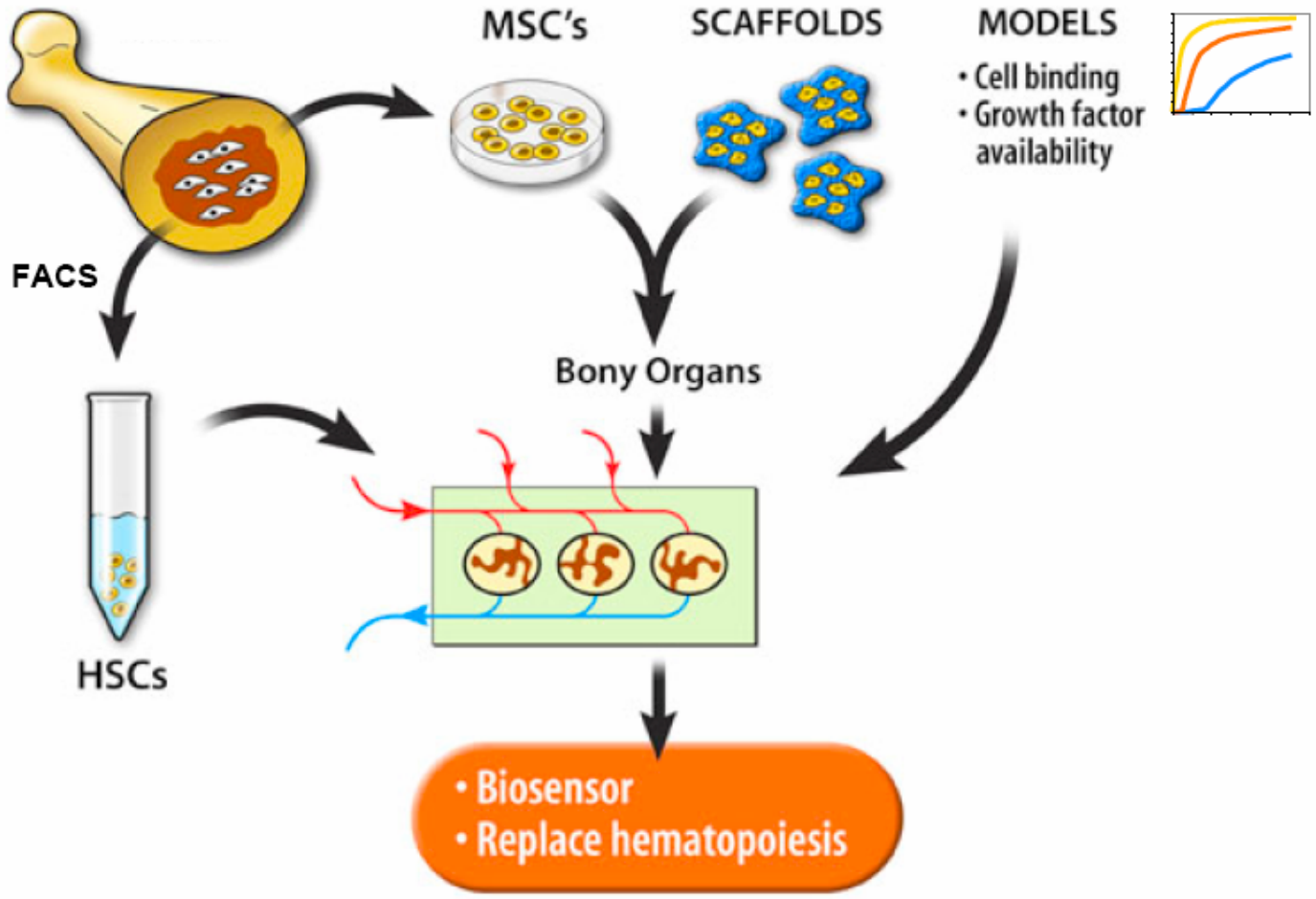
  (marrow most sensitive organ to cytotoxic signals)

- reconstitution of the hematopoietic system of soldiers

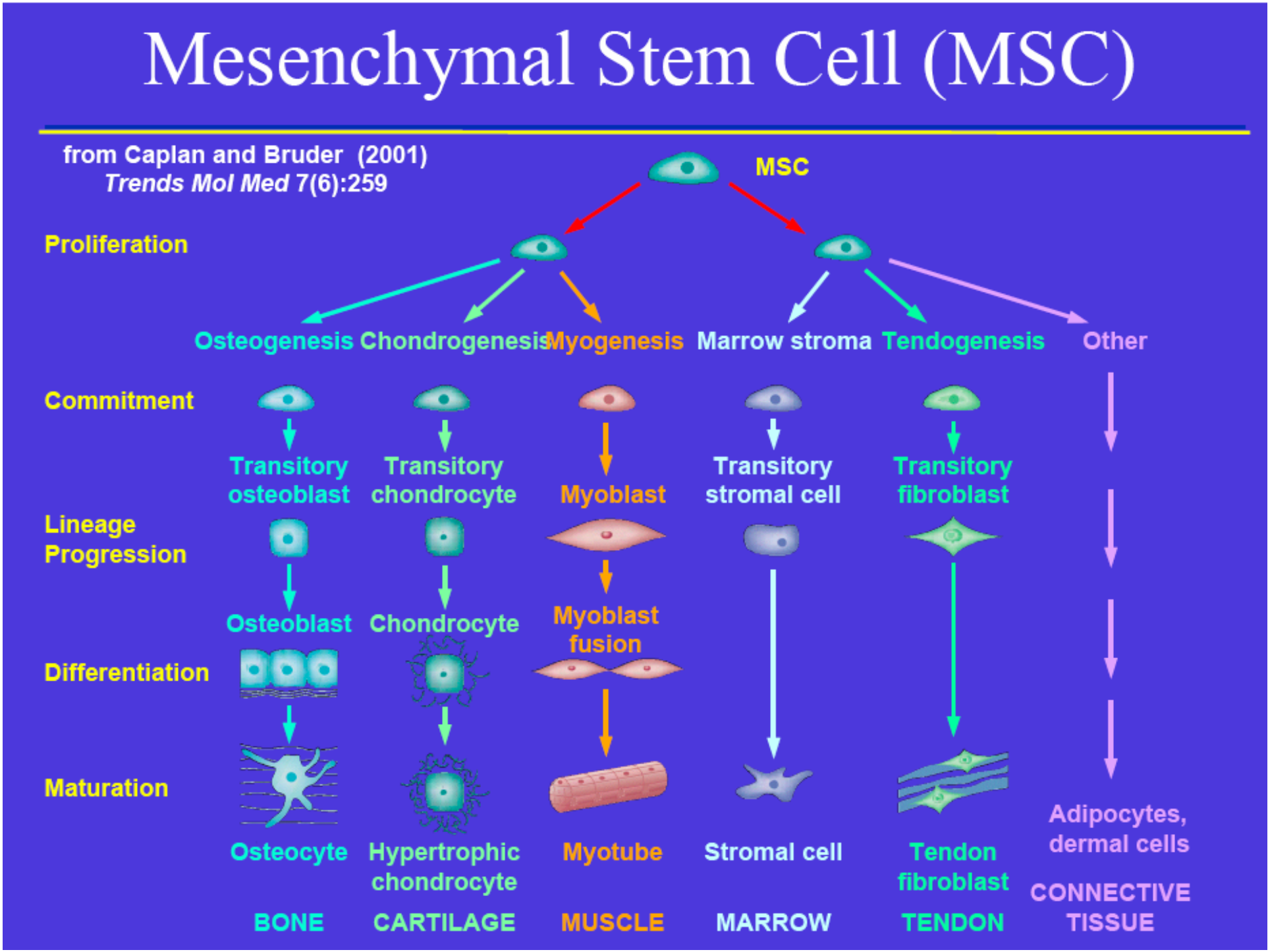
- reconstruction of large bony defects

- template for other organs?

STRATEGY


MSC's
SCAFFOLDS
MODELS
• Cell binding
• Growth factor availability
FACS
Bony Organs
HSCs
• Biosensor
• Replace hematopoiesis

Mesenchymal Stem Cell (MSC)



MSC Biology: Immortalization & Differentiation

Human telomerase reverse transcriptase (hTERT)

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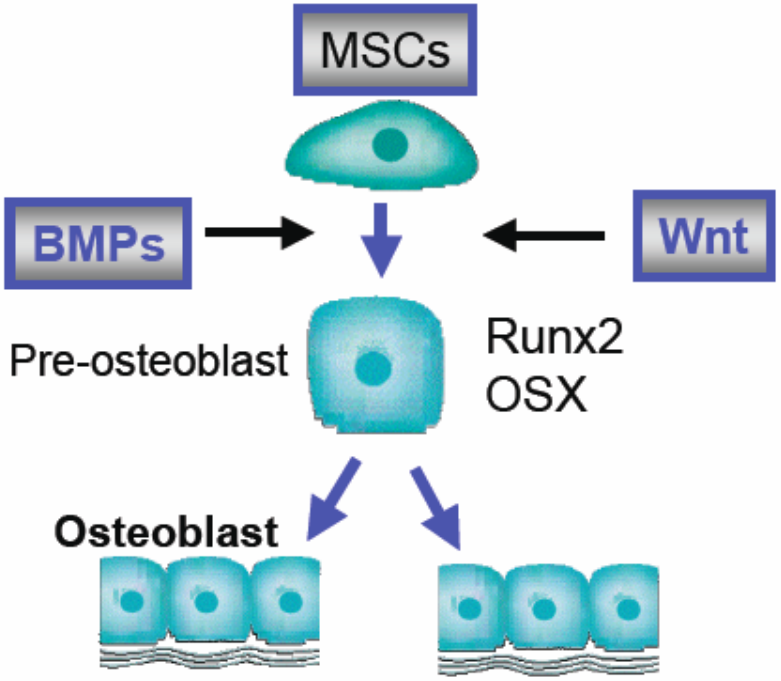
The Life Span of Cells ↑



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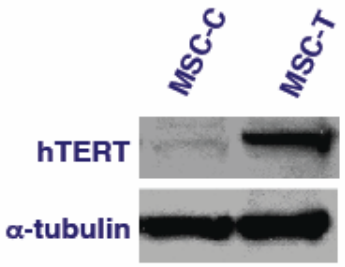

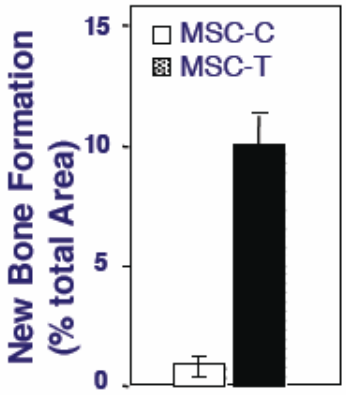

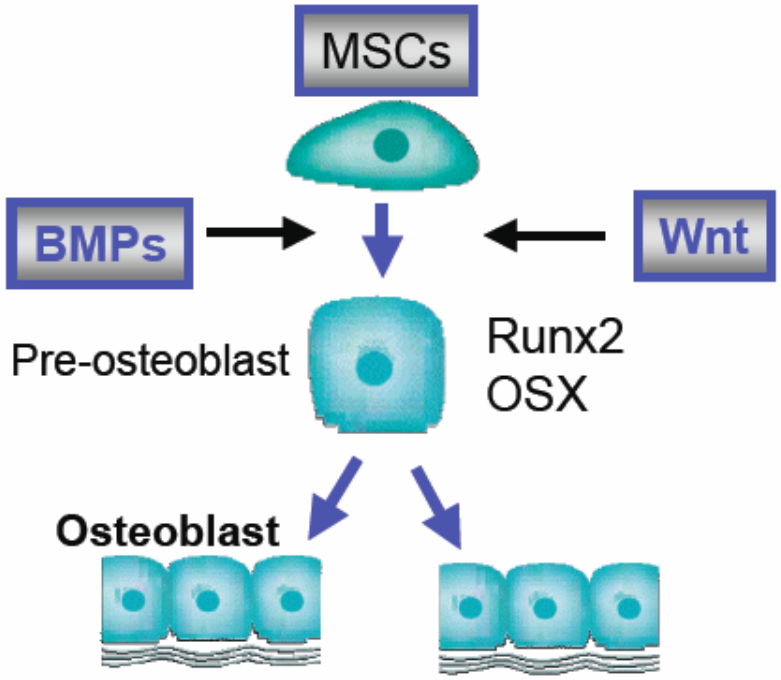

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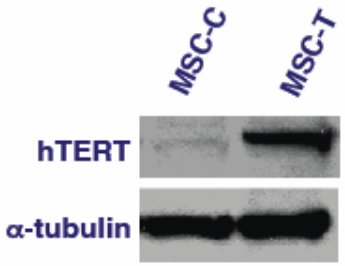
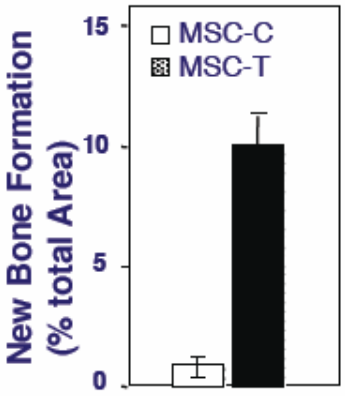
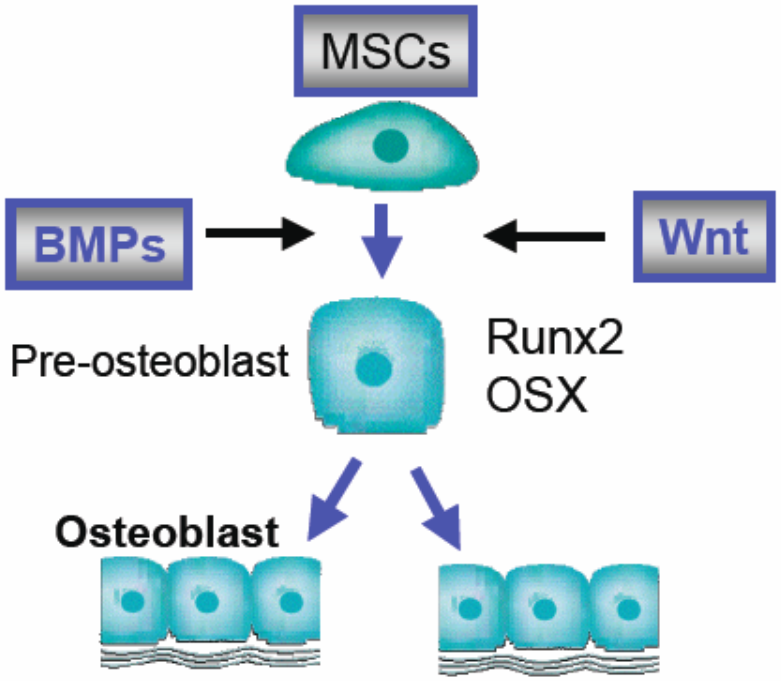
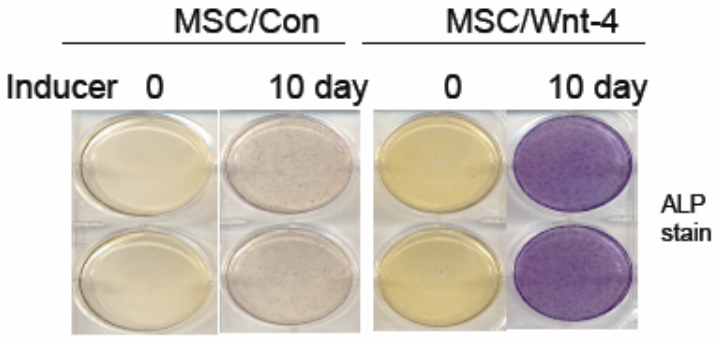
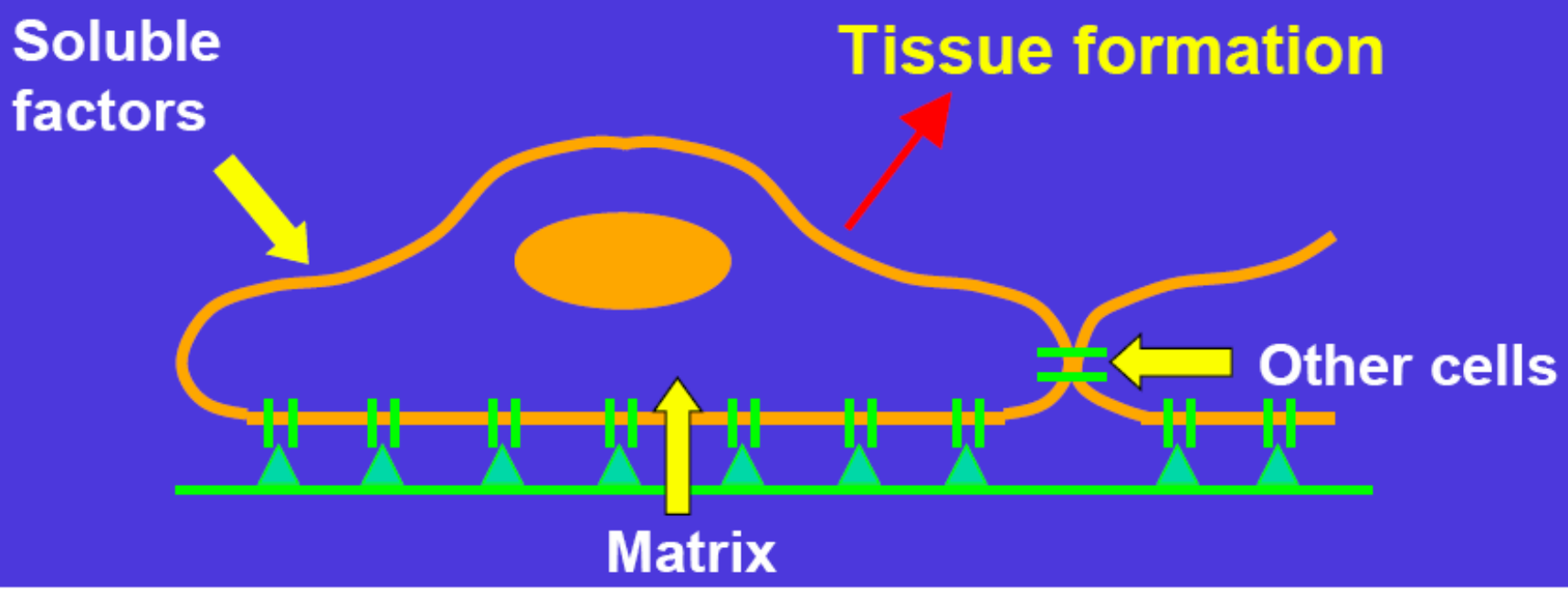








Bio-Based Material Design



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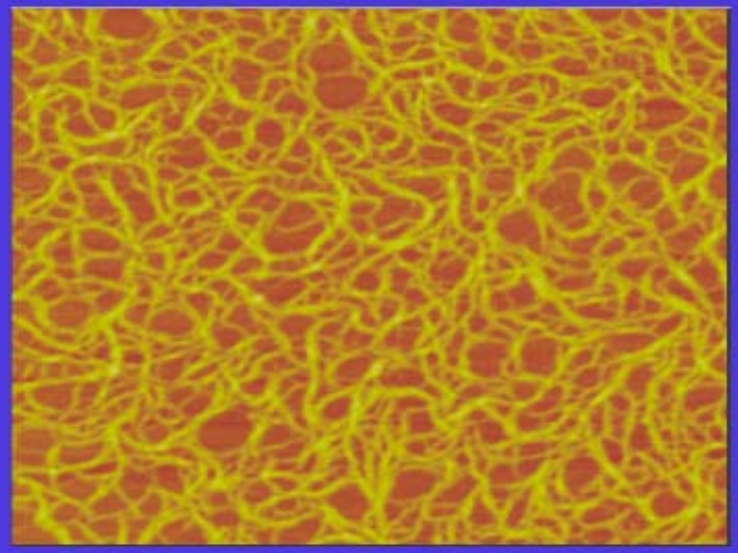
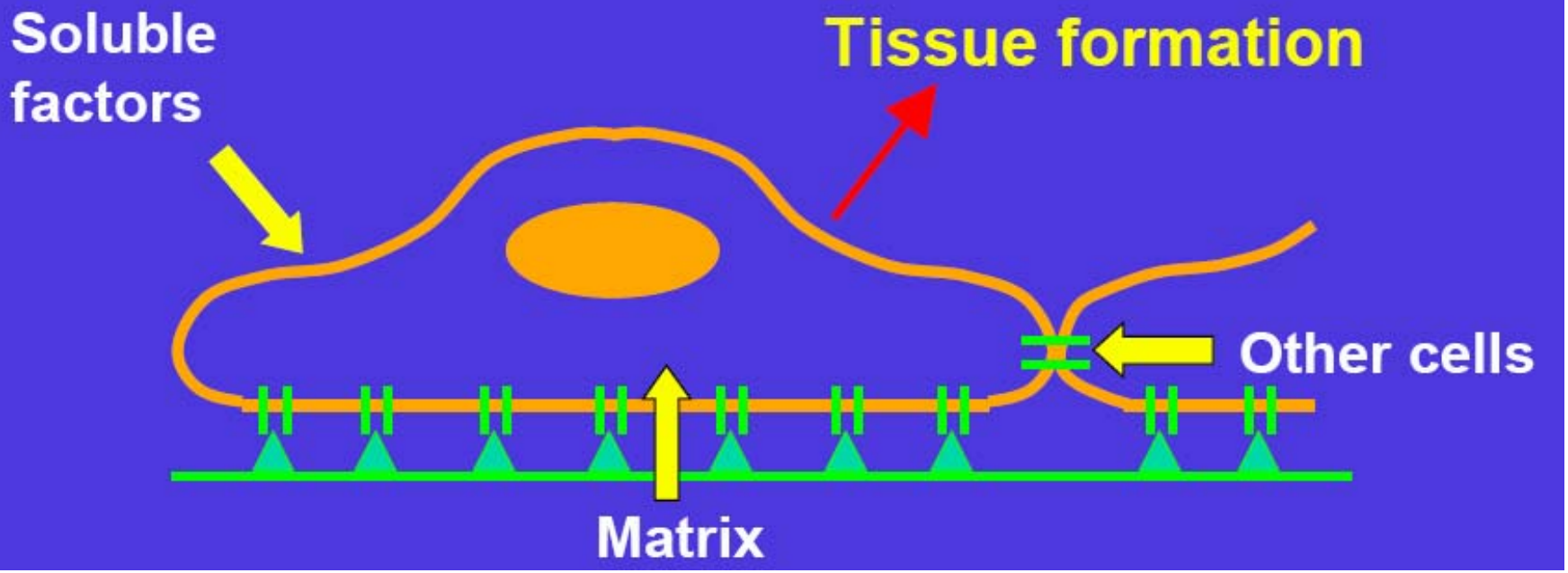
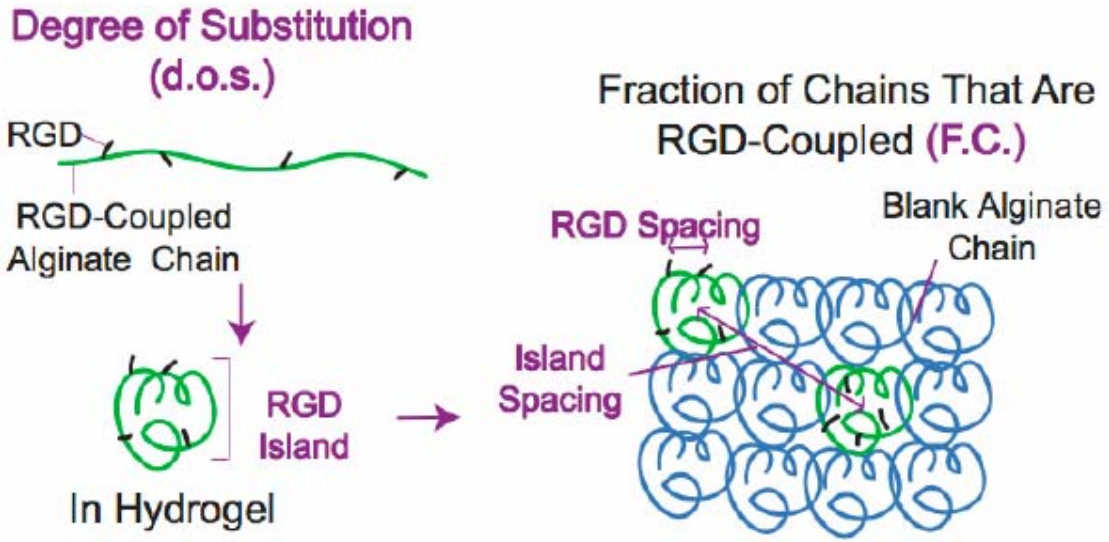


Nanoporous gels

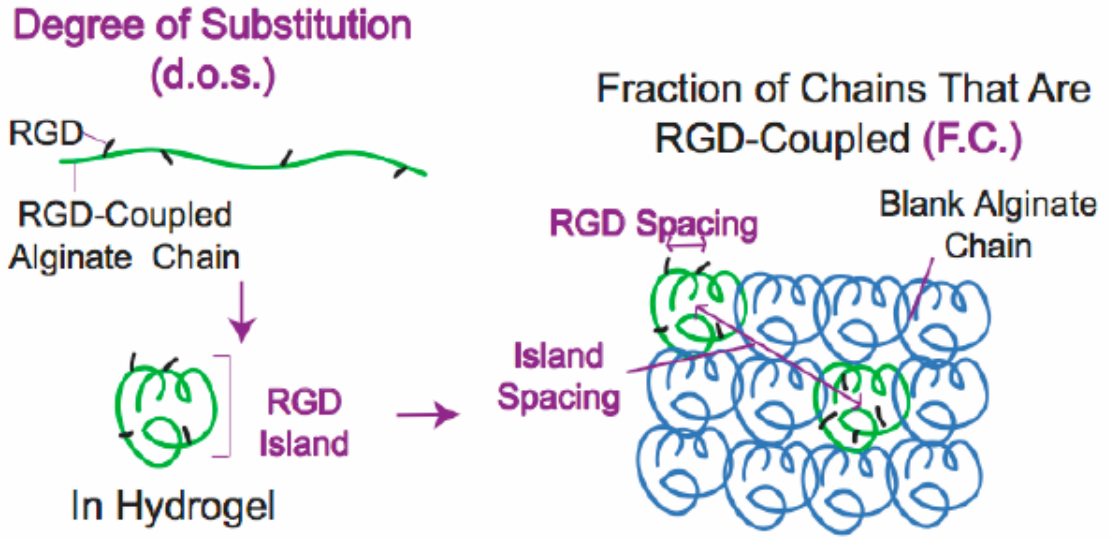


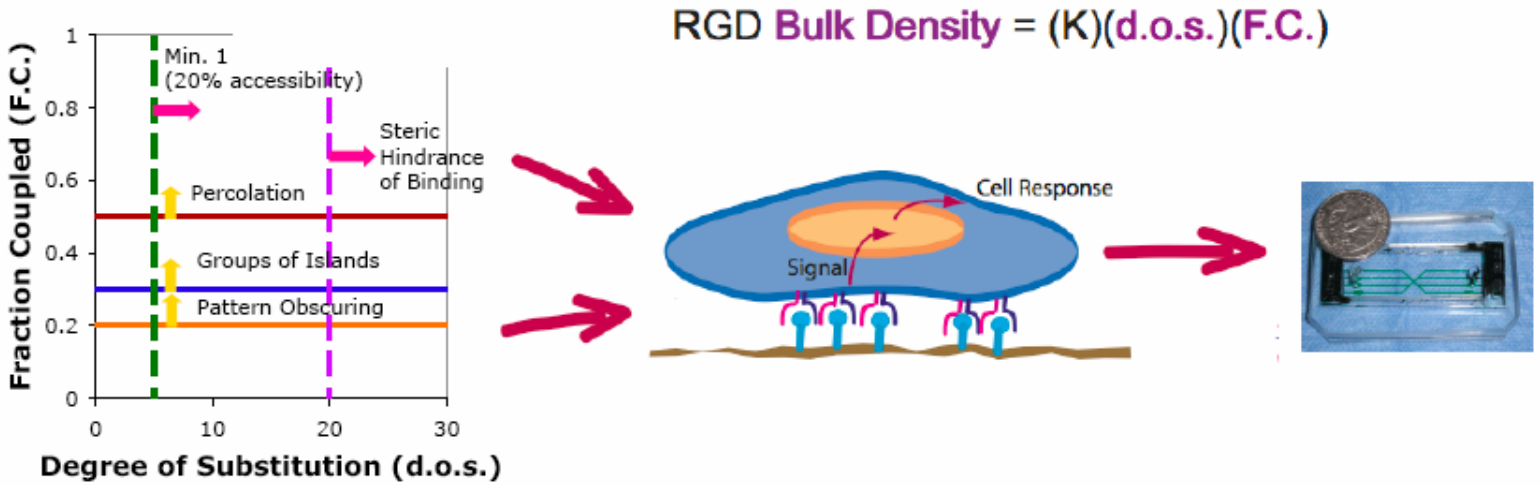
Nanopatterned Materials



RGD Bulk Density = (K)(d.o.s.)(F.C.)

Nanopatterned Materials

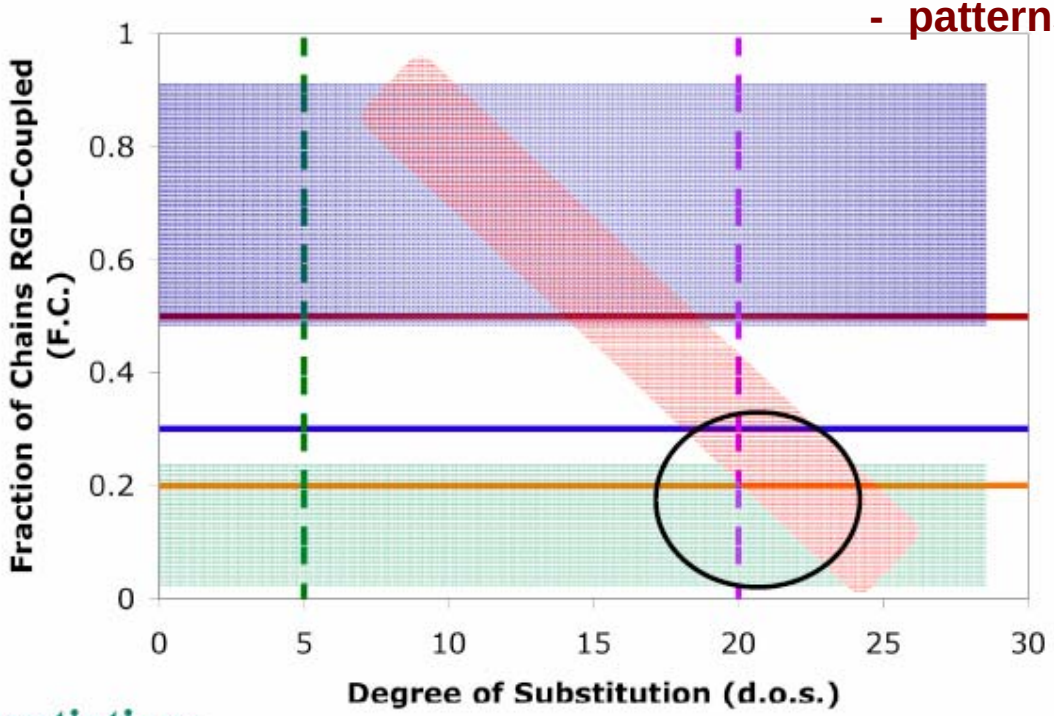




OPTIMAL NANOPATTERNS

•FAK & Spreading:
- –Groups of islands best
- –F.C. > 0.5 (percolation)

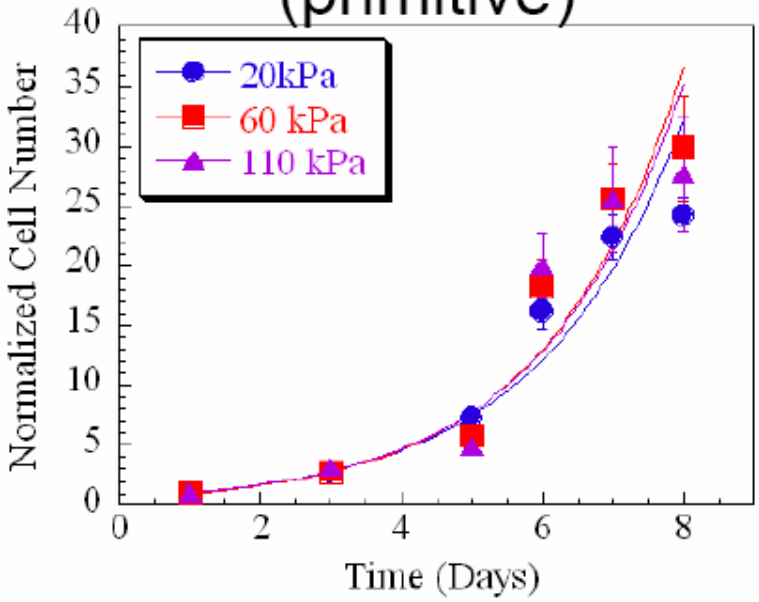
- **Proliferation:**
  - – Intermediate bulk density best
  - - patterns don't matter



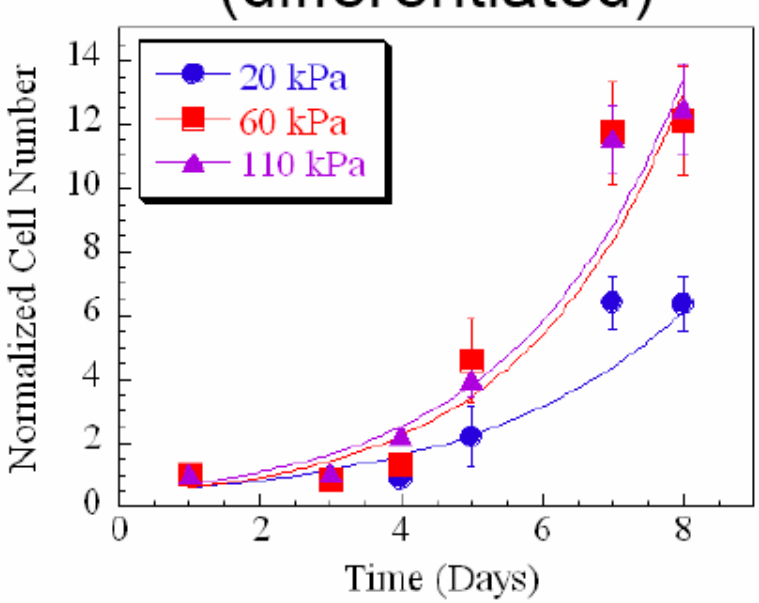
- **Differentiation:**
  - – Single islands best
  - – Maximum at 0 > F.C. < 0.1

DIFFERENT RESPONSE: STEM CELLS

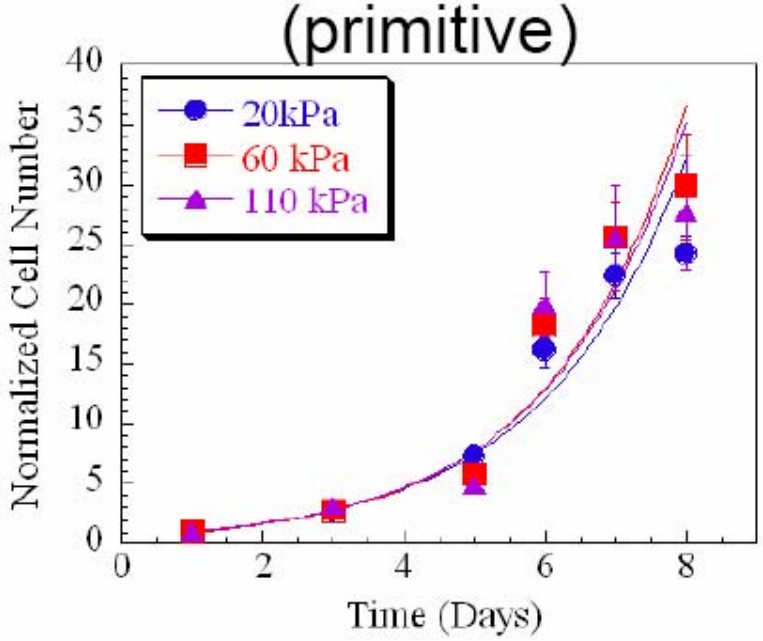
(primitive)

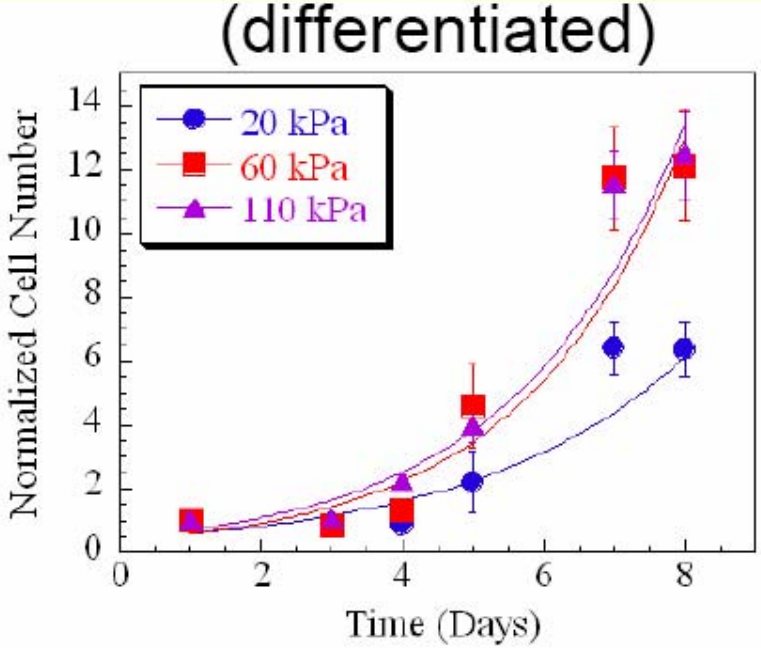


(differentiated)

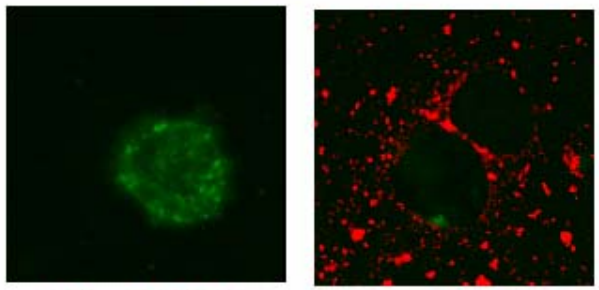


DIFFERENT RESPONSE: STEM CELLS

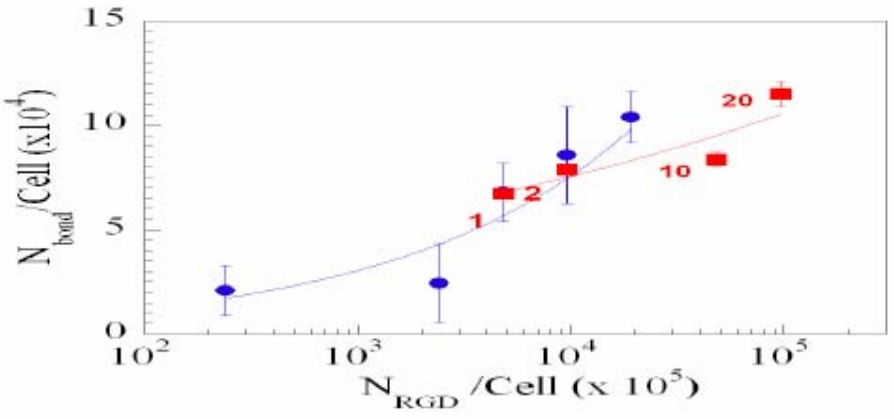




Imaging w/ FRET

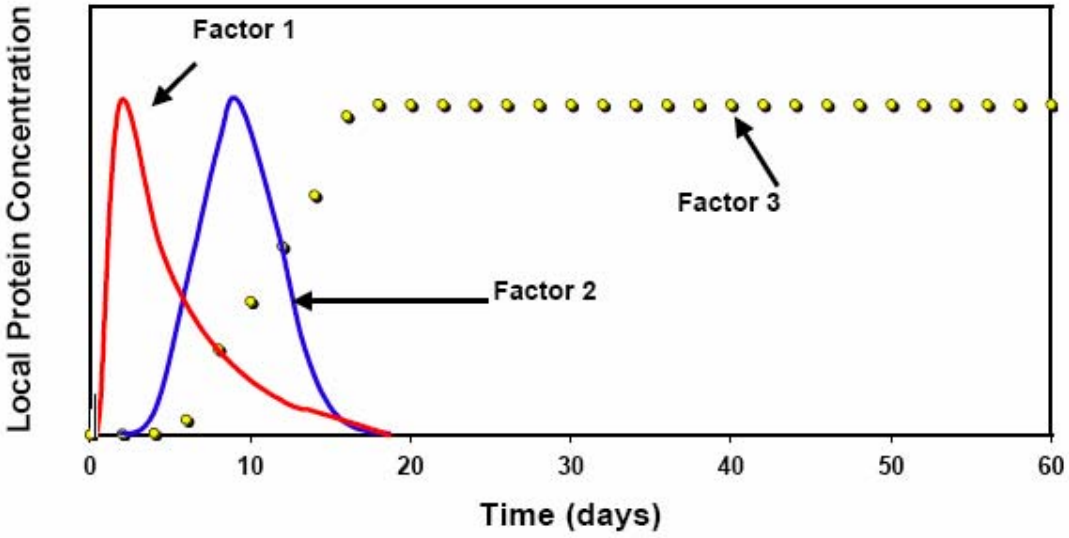


[No RGD] [DS 10 RGD]

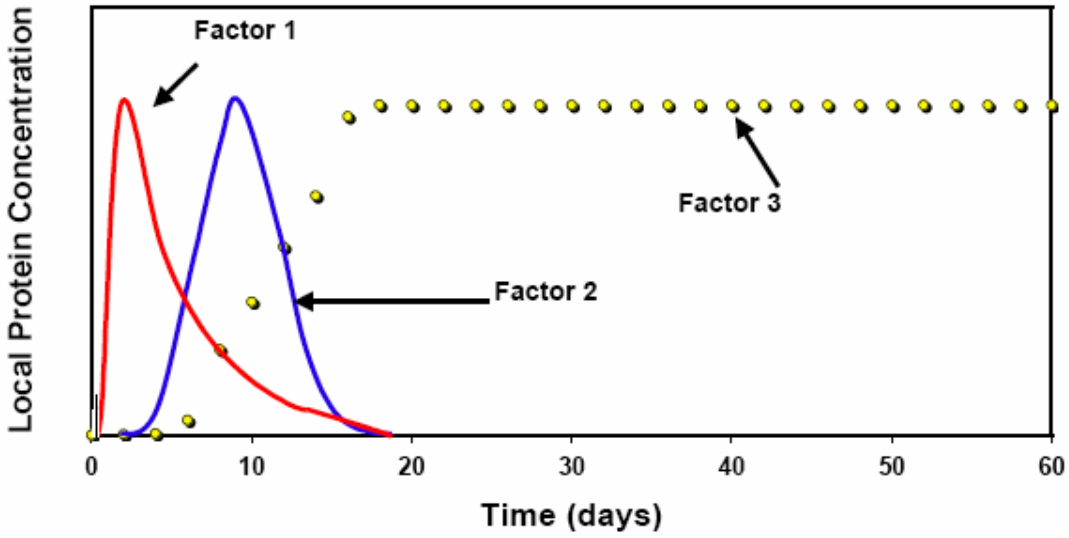


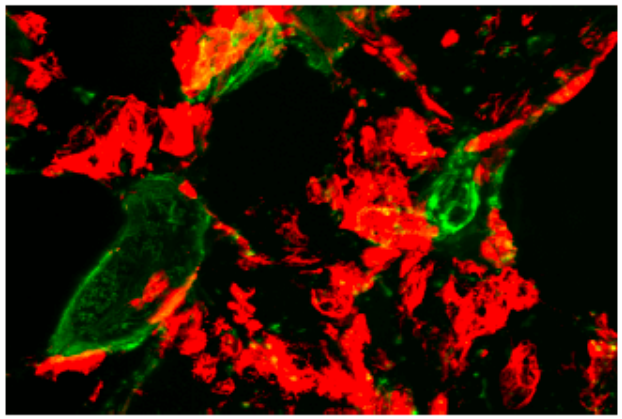
(Kong et al., PNAS 2006)

DYNAMIC MORPHOGEN PRESENTATION

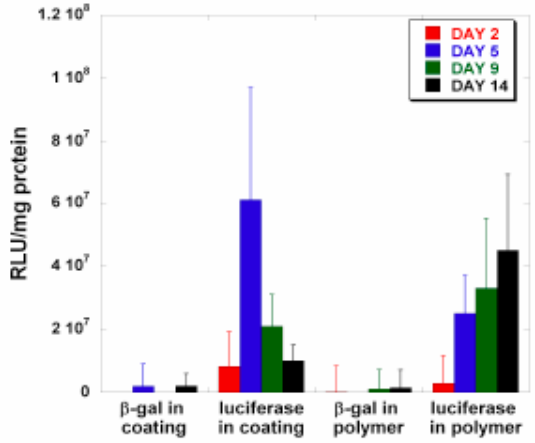


DYNAMIC MORPHOGEN PRESENTATION





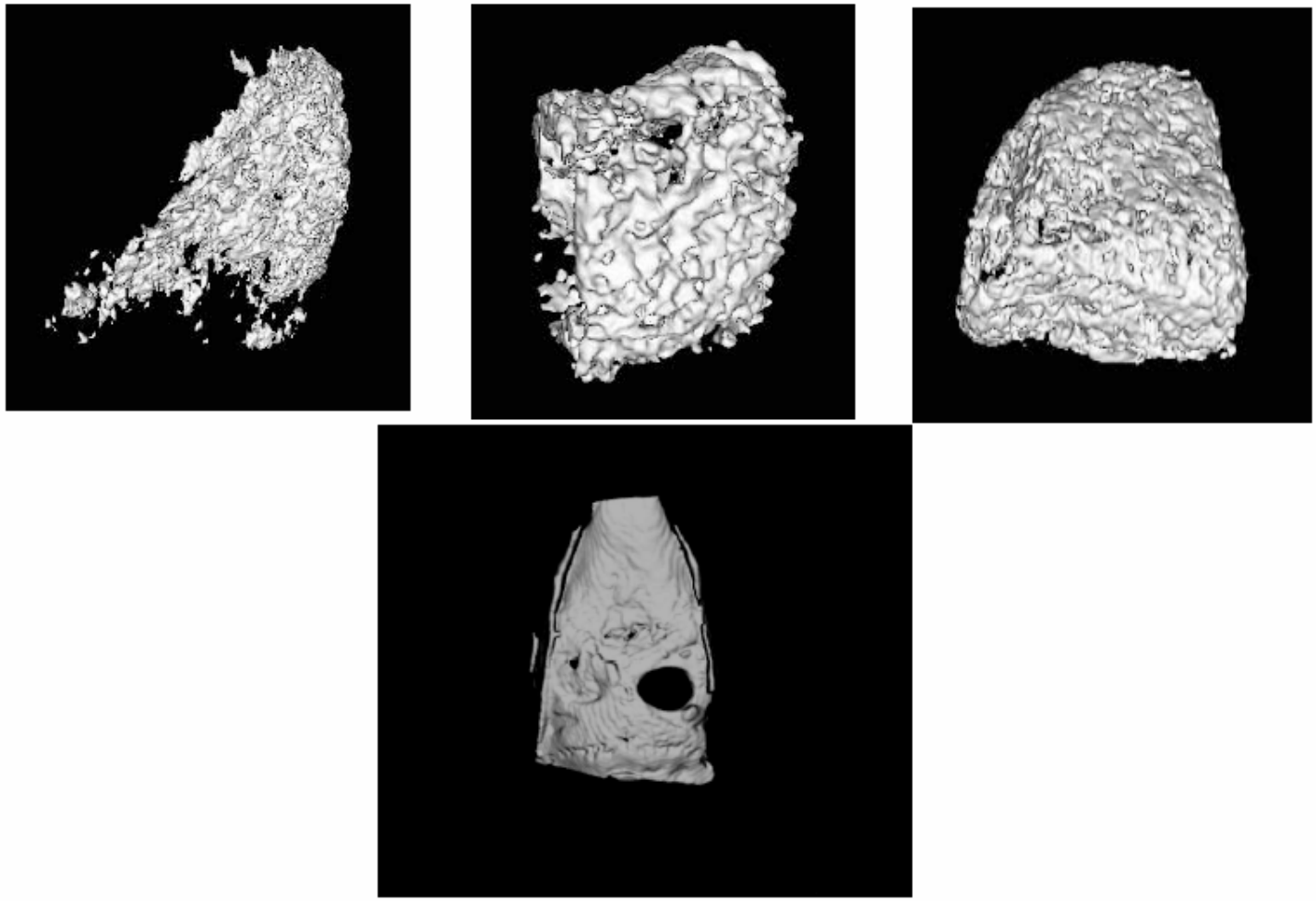
(recombinant morphogens)



(local gene therapy)

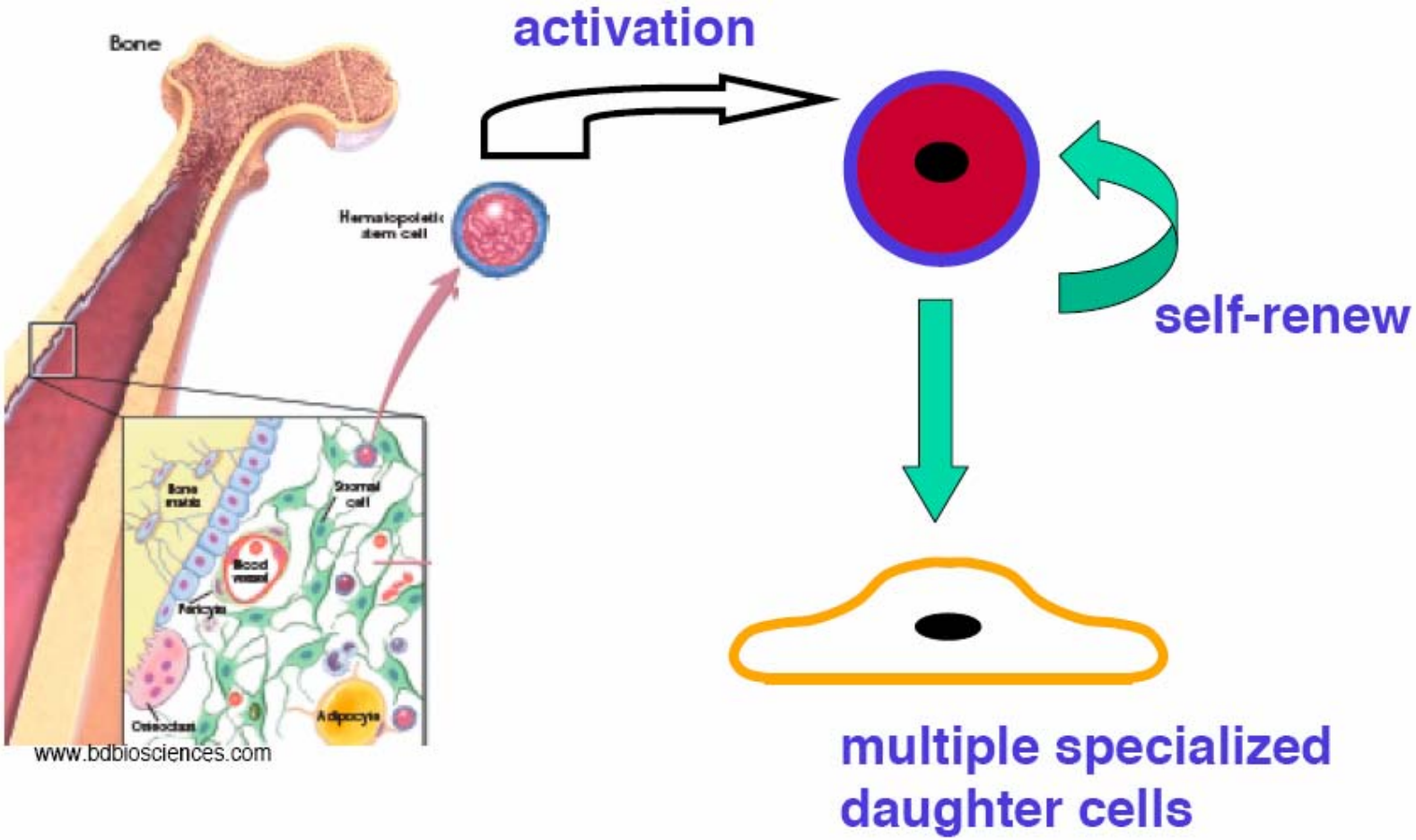
Rapid Bone Formation *In Vivo*

3 8 15 weeks

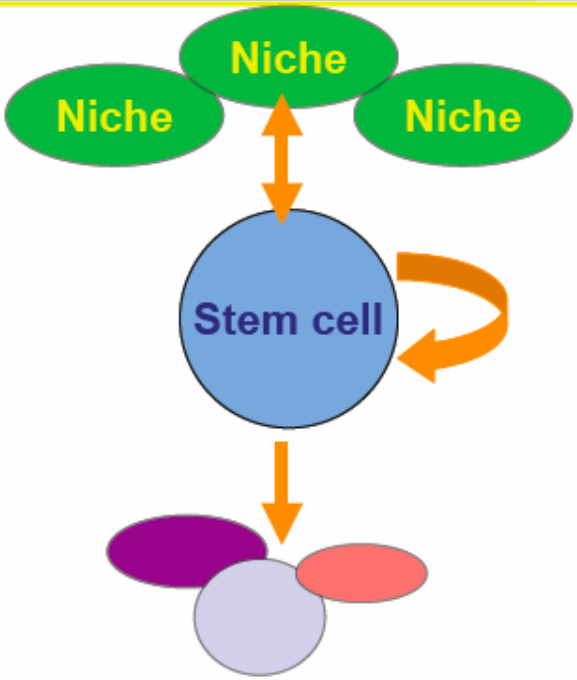


(Huang et al., *JBMR* 2005; Kaigler et al., *JBMR* 2006)

HEMATOPOIETIC STEM CELL (HSC) NICHES

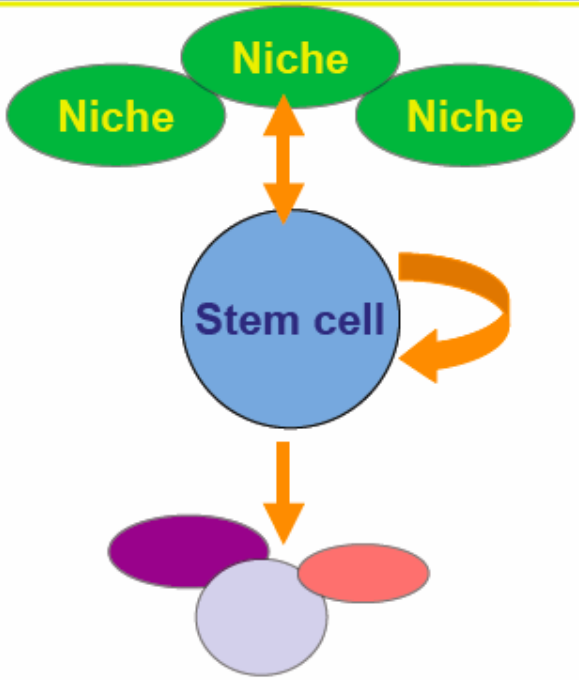


HSC PURIFICATION & LOCALIZATION



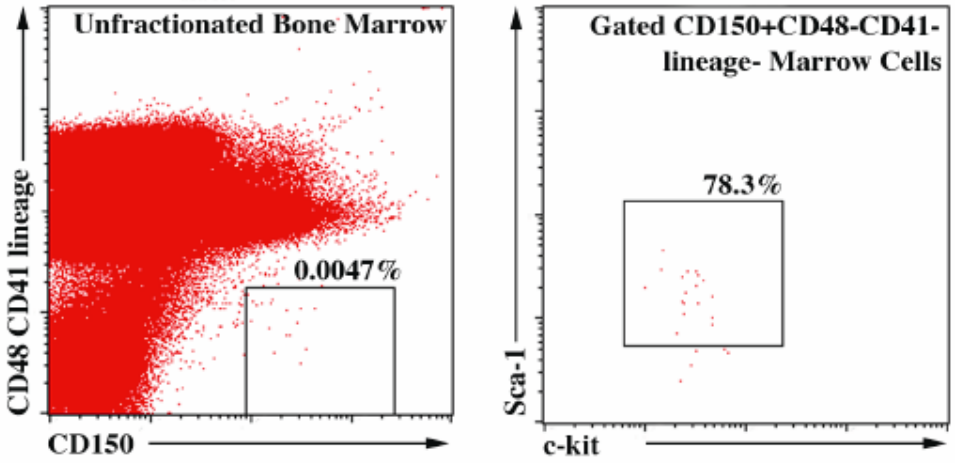
Identifying HSCs New markers that greatly enhance the purification of HSCs (Cell 121: 1109; Blood 106: 903)

HSC PURIFICATION & LOCALIZATION

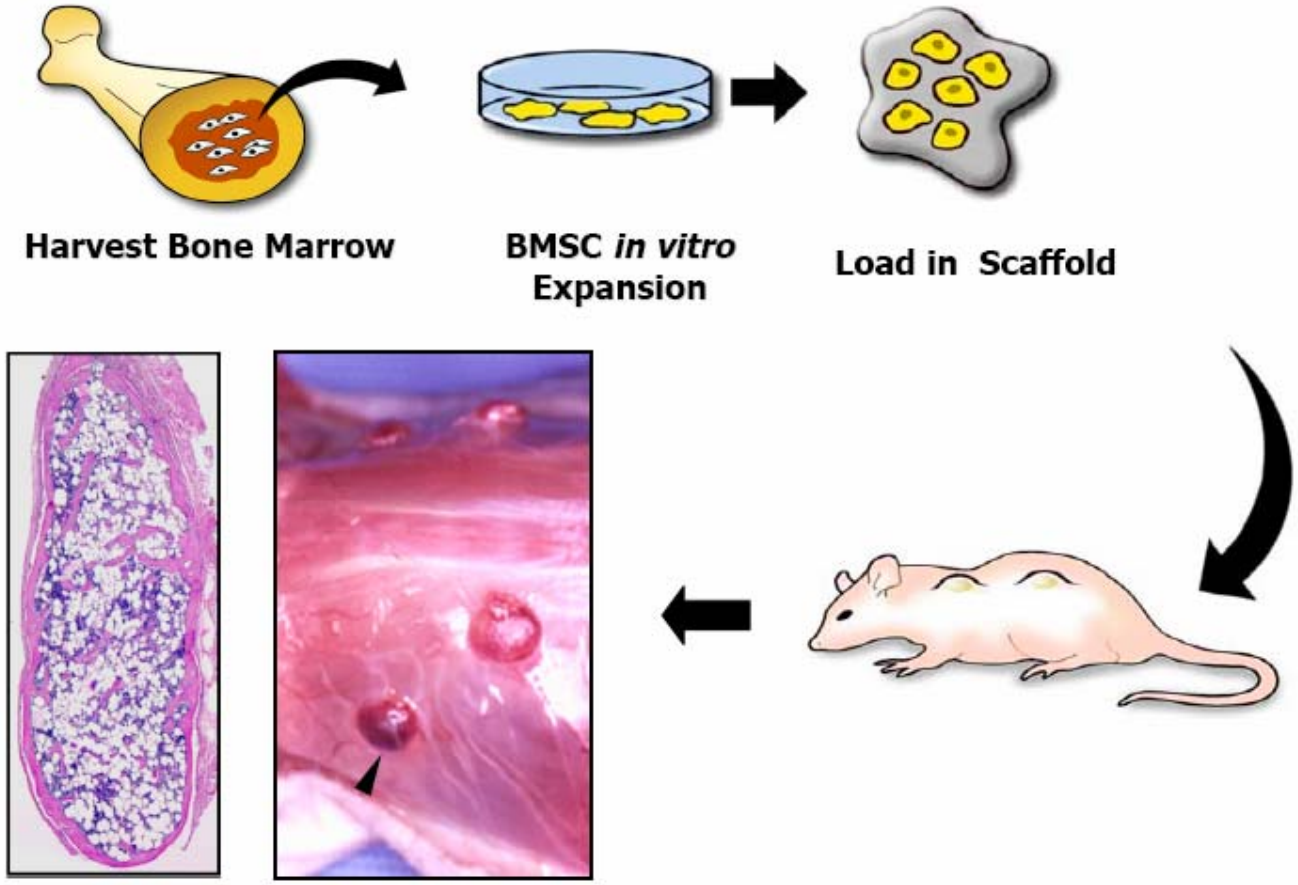


Identifying HSCs New markers that greatly enhance the purification of HSCs (Cell 121: 1109; Blood 106: 903)

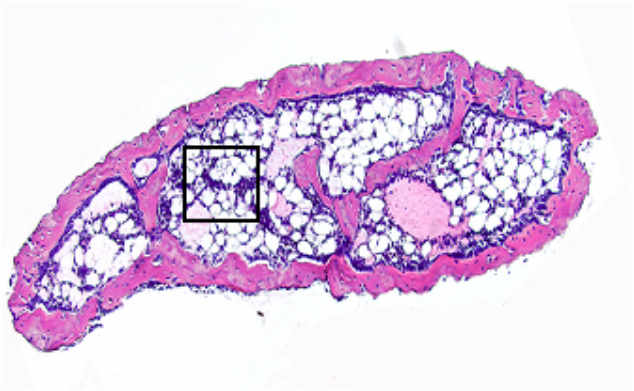
45% of single $CD150^{+}CD48^{-}CD41^{-}$ cells give HSC activity in irradiated mice

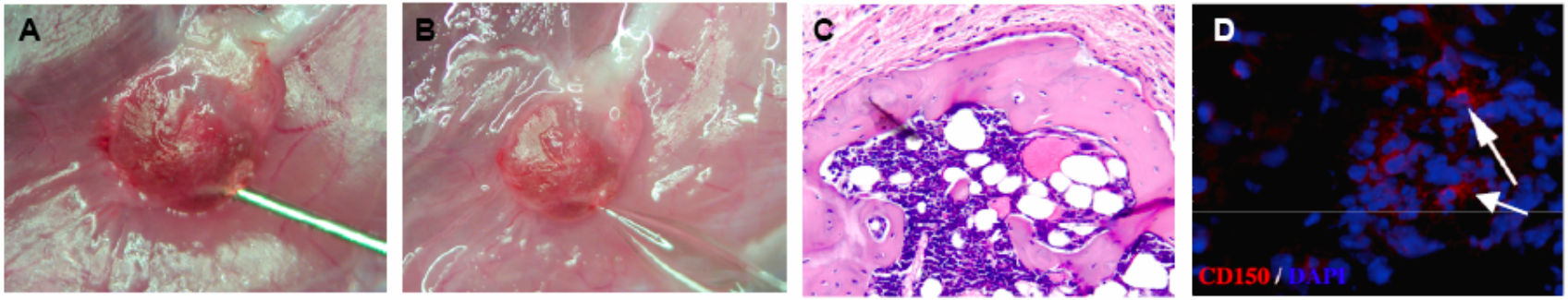


MODEL SYSTEM



Identification of HSCs in Tissue Engineered Bone

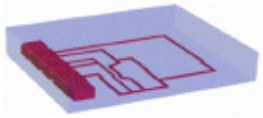




HSCs identified with SLAM code markers

Microfluidic Circulatory Support System

Flo Chips	PinFlo Pump	PinFlo Software

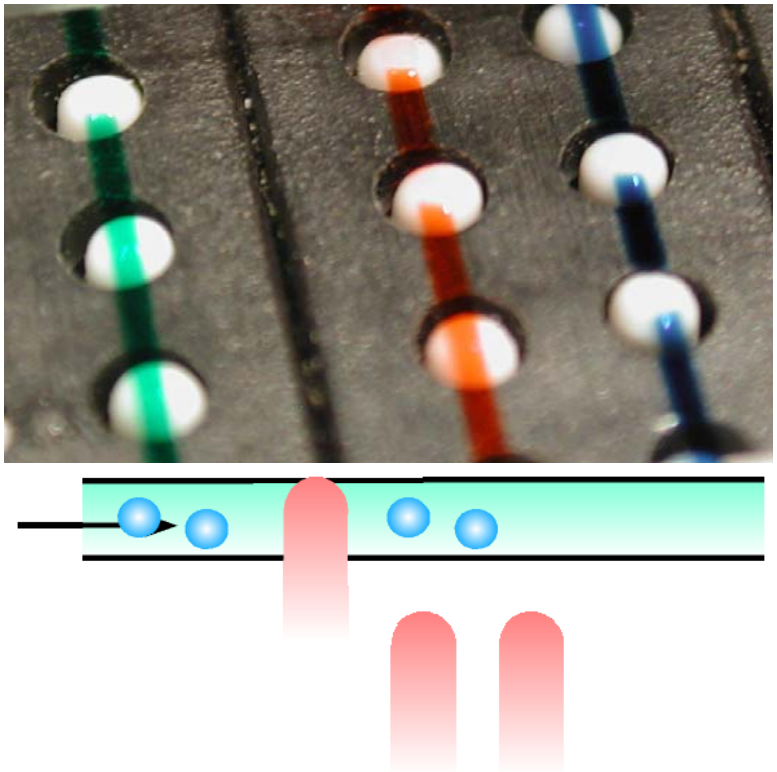


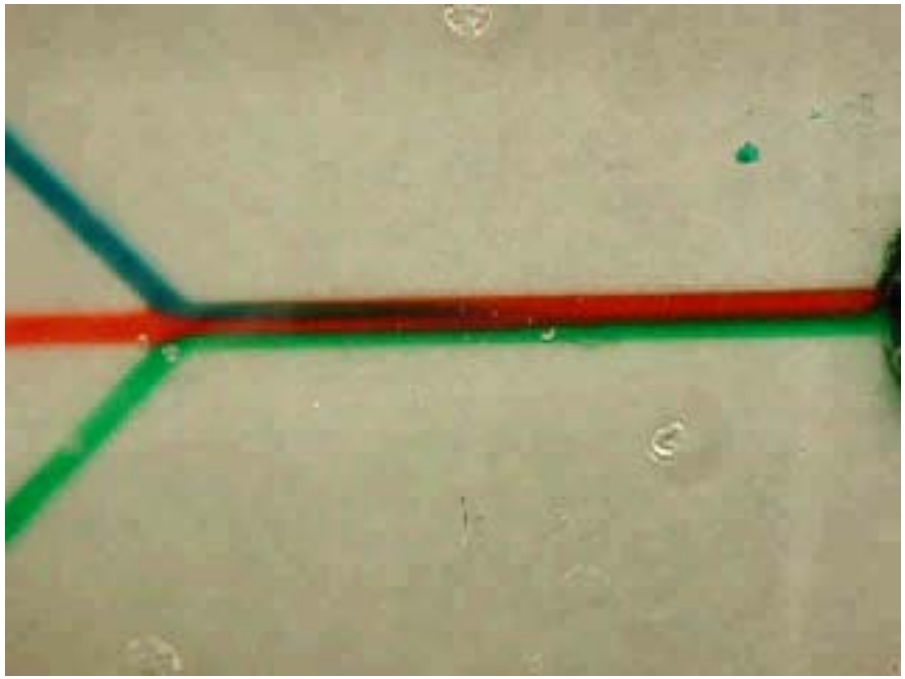
- Inexpensive
- Biocompatible
- Flexible Layouts
- "Vasculature"

- Sophisticated Controls
- No Tubing
- "Heart"

- Programmable
- Experiments on Demand
- "Brain"

Programmable Microfluidics

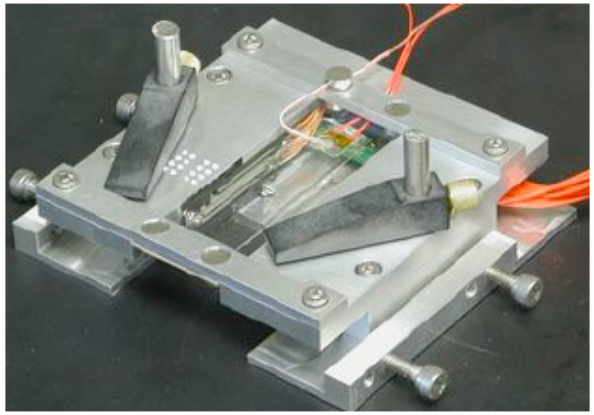




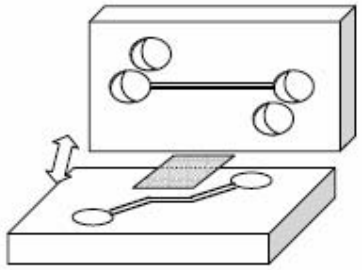
On-Demand Microfluidic Control For Next-Generation Cell Culture and Microfluidic Tissue Engineering.

Device Status

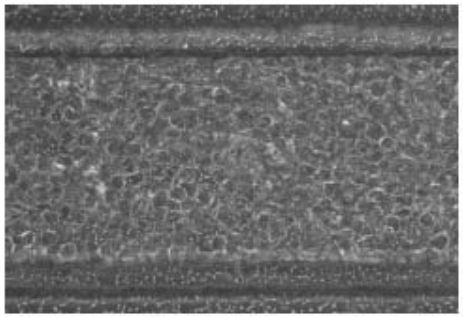
- Snap on system
- Real-time imaging
- On-chip heating
- Special Media/Channel
- Totally stand alone 2hrs

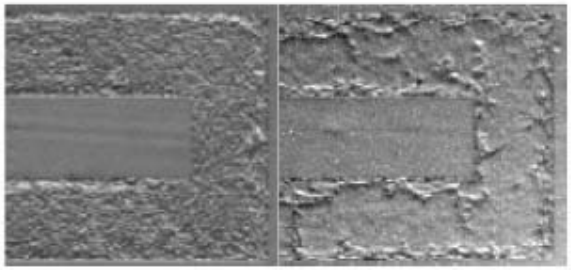


Mineralization MC3T3 in Alginate



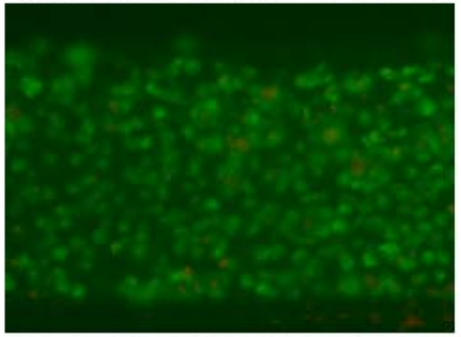
Cells & Gels
In Channels

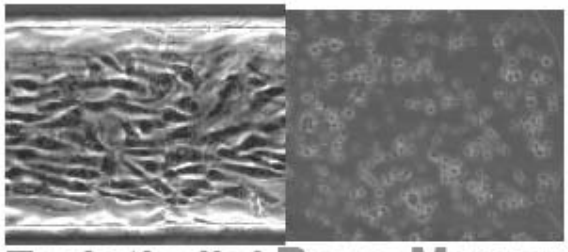




C2C12 MC3T3

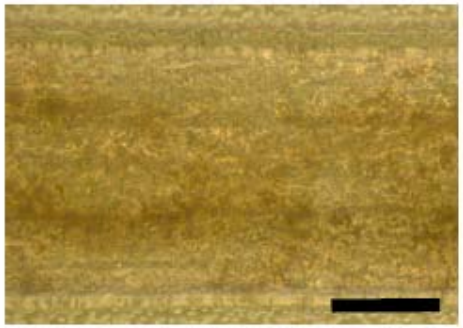
Cells Alive





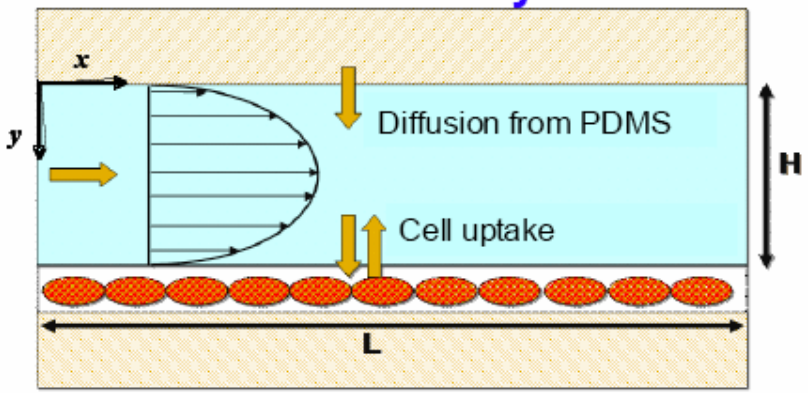
Endothelial Bone Marrow

Mineralization

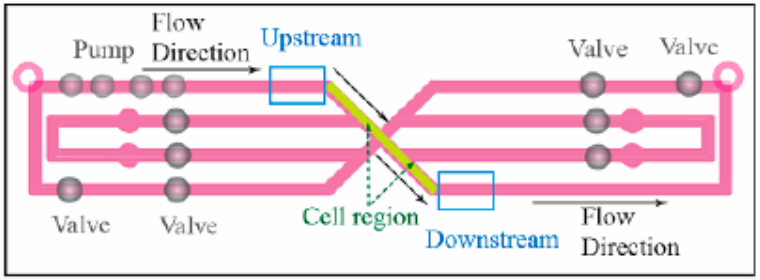


Oxygen Quantification in Microbioreactor

Model Geometry

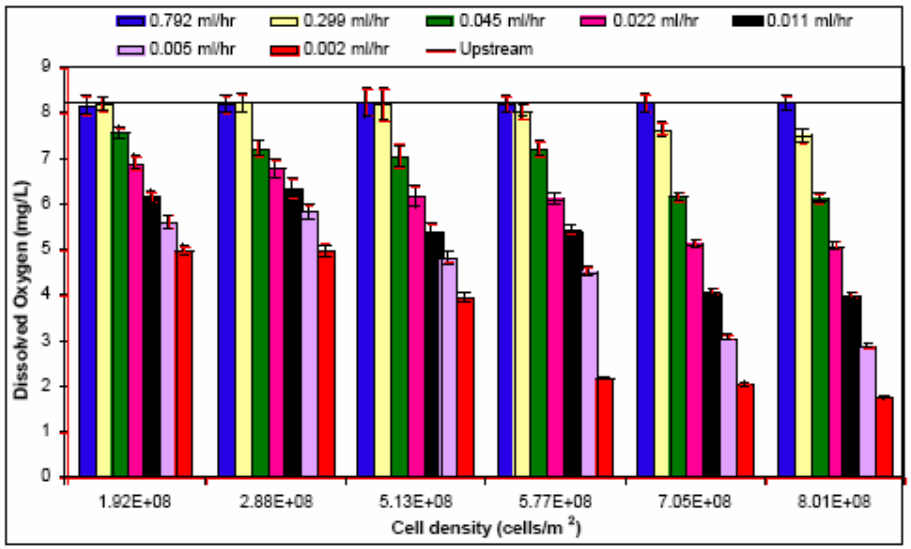


Oxygen Measurement Technique

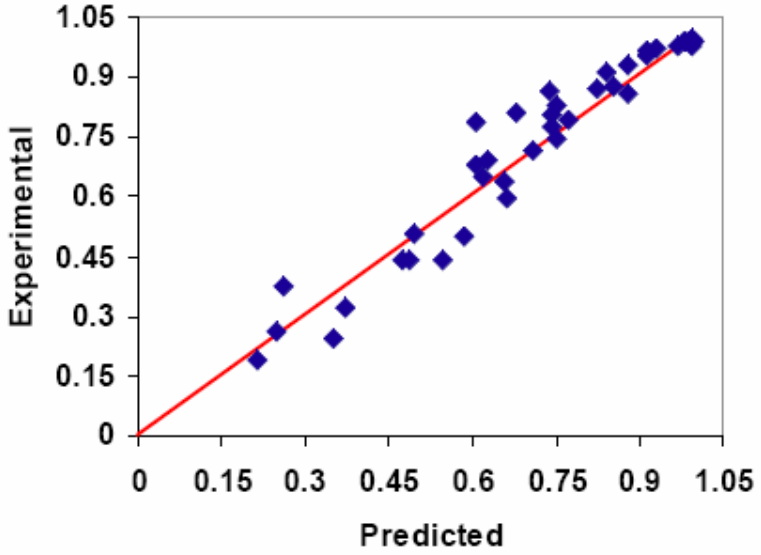


Fluorescence intensity measurements with ruthenium based dye

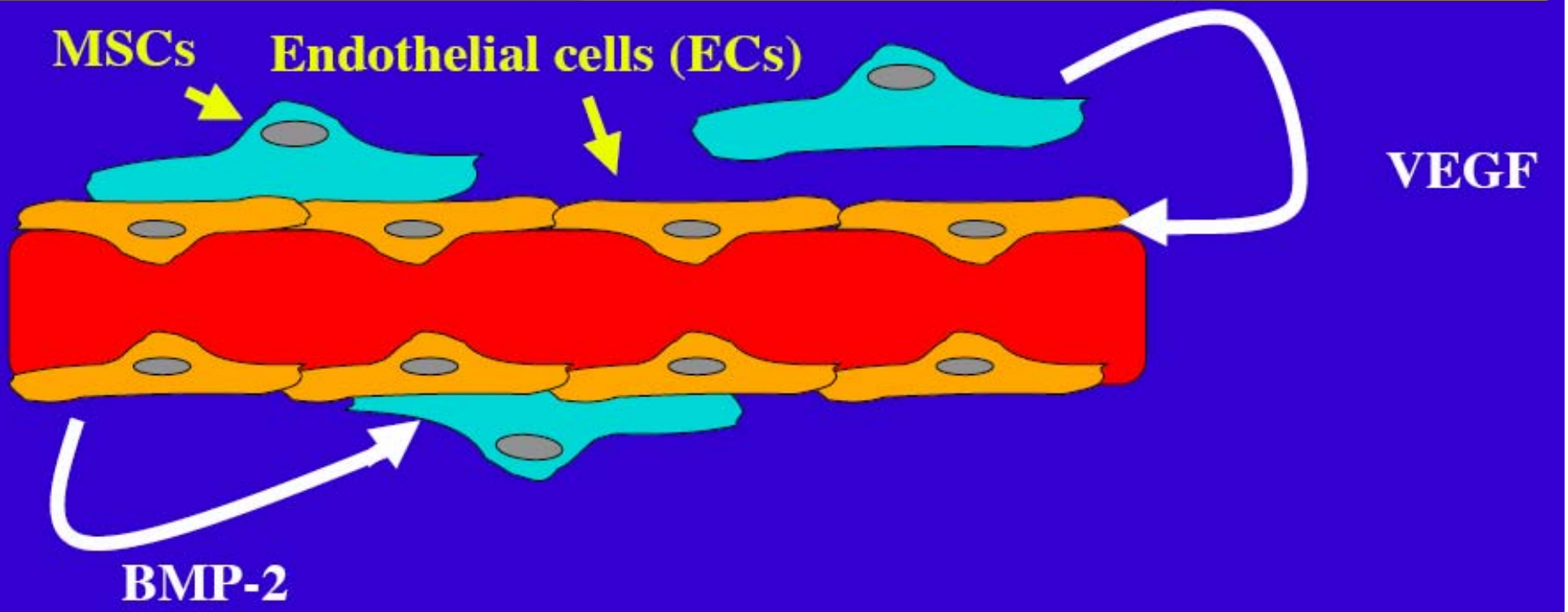
Oxygen Gradients in Device



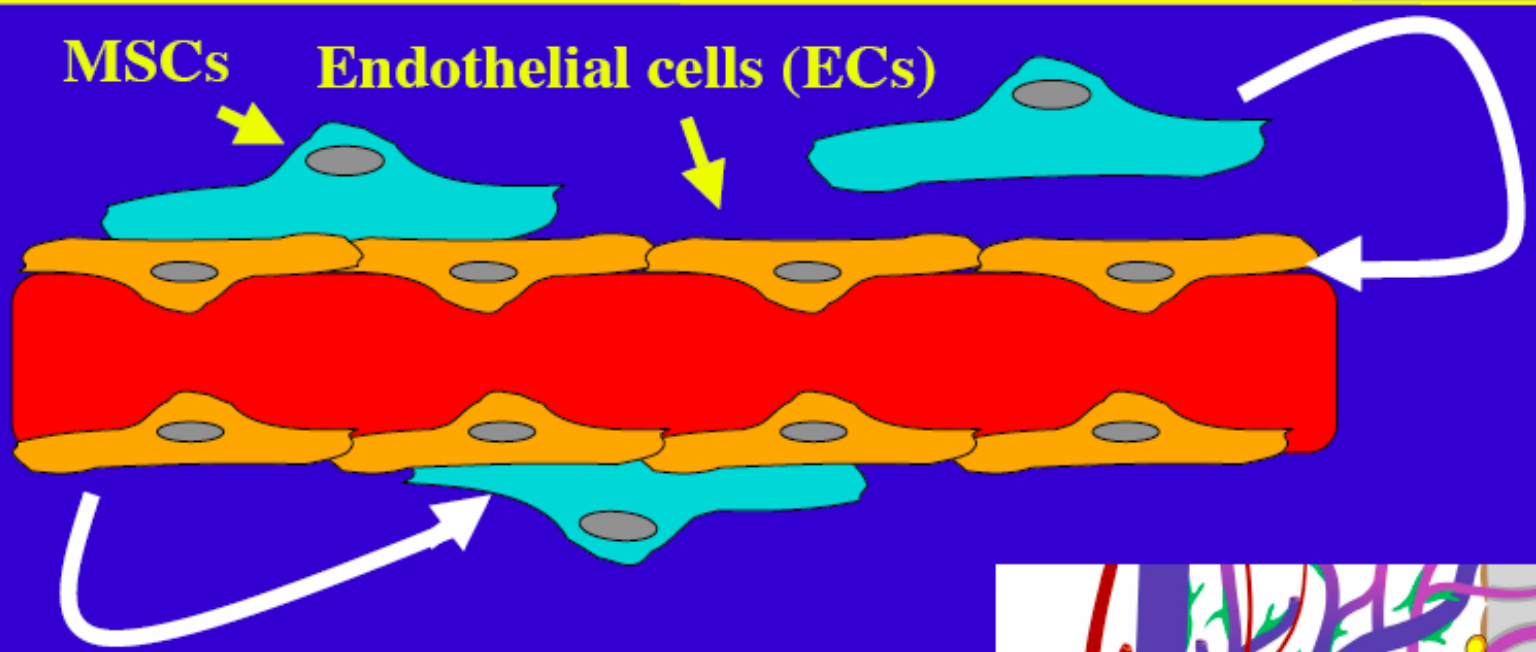
Model Predictions vs. Experimental Data

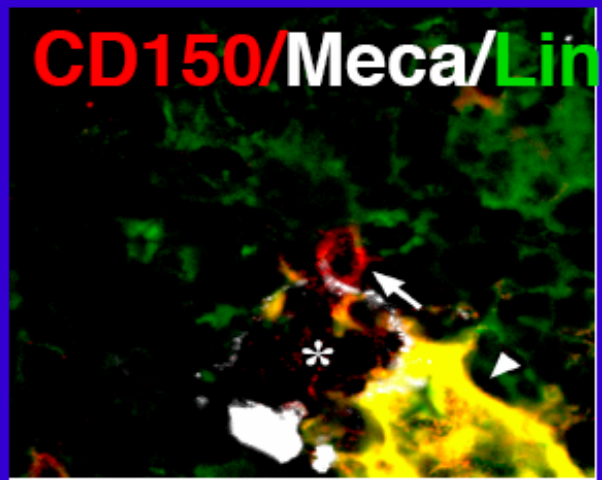


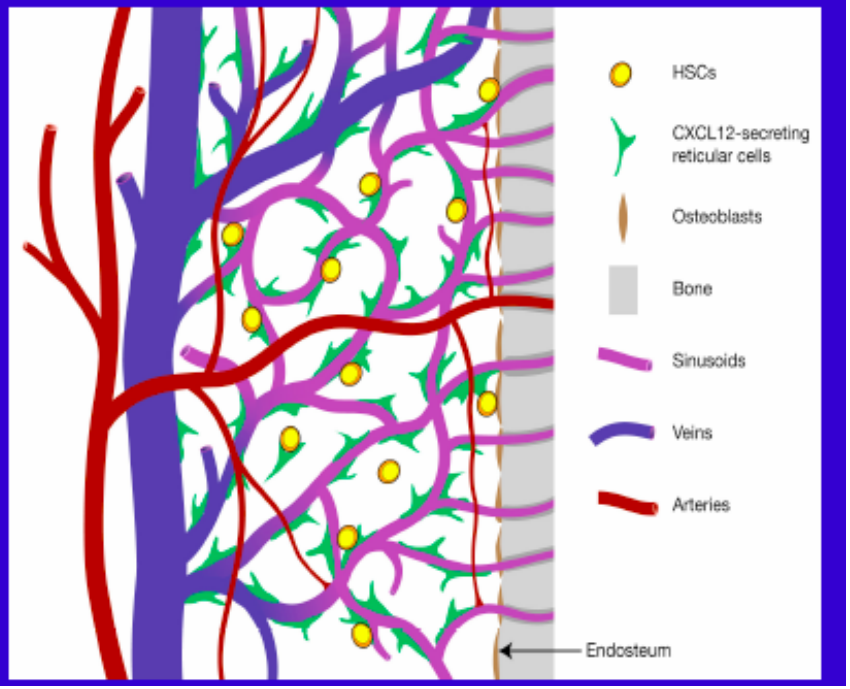
CROSSTALK WITH VASCULAR CELLS



CROSSTALK WITH VASCULAR CELLS







Engineering Hybrid Bony Organs